EFFECT OF *CYMBOPOGON CITRATUS* (LEMON GRASS) AQUEOUS EXTRACT ON BLOOD GLUCOSE, BODY WEIGHT AND LIVER, KIDNEY AND PANCREAS REDUCED GLUTATHIONE CONCENTRATION ON NORRMAL AND STREPTOZOTOCIN-INDUCED WISTAR RATS

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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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JANUARY, 2023

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IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (B.Sc) IN BIOCHEMISTRY

JANUARY, 2023

CERTIFICATION

This is to certify that the research work “aqueous extract effect of *Cymbopogon citratus* (lemongrass) on blood glucose, body weight and liver, kidney and pancreas reduced glutathione concentration on norrmal and streptozotocin-induced Wistar rats was carried out and written by **OISAMOKHAI AVBIEGBE CYNTHIA (MAT NO: LSC1705210) in** the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin city, in partial fulfilment of the award of Bachelor of Science, (B.sc) degree in Biochemistry.

____________________ ____________________

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PROF. (MRS) K.E. IMAFIDON **DATE**

(Head of Department)

DEDICATION

This work is dedicated to God my father, who is my source, my goal and my end.

ACKNOWLEDGEMENT

My profound gratitude goes to Almighty God for His love, guidance and wisdom shown to me throughout my B.Sc. programme and my life in general. My gratitude also goes to my supervisor Prof. (Mrs) R. I. Nimenibo-Uadia for her assistance, supervision and corrections throughout my project. I sincerely appreciate my lovely parents, Mr and Mrs Ojo Erimiakhena, my caring siblings, Dr Ken Oisamokhai, Engr. Judith Oisamokhai and God'swill Oisamokhai for their financial, moral and spiritual support throughout my studies. Big thanks goes to my man of God, Rev P.N utomi for his fatherly care, Bishop Chris Phill, Bro chukwuyem onyekpe, Sis joy Iruamaka , Sis Bernice Special thanks to Dr. O.C Ugbeni. F.O. Obi, Prof.(Mrs) E.S. Omoregie, Prof. (Mrs) M.A. Adaikpoh, Prof. (Mrs) B.O. Agoreyo, Prof. N.P. Okolie, Prof. (Mr) Uadia and Dr. Osahon Abu whom without their guidance, I would not have made it this far. Finally, my sincere appreciation goes to my friends Ezeagu Prisca, Idubor Precious, all my project members and all adaptogen class for their encouragement and support.

TABLE OF CONTENTS

Table of Contents

CERTIFICATION ii

DEDICATION iii

ACKNOWLEDGEMENT iv

TABLE OF CONTENTS v

ABSTRACT vii

CHAPTER ONE 1

INTRODUCTION AND LITERATURE REVIEW 1

1.1 INTRODUCTION 1

1.2 AIMS AND OBJECTIVES 3

1.3 LITERATURE REVIEW 4

1.4 TAXONOMY 4

1.5 DIABETES MELLITUS 5

1.6 THE KIDNEYS 9

1.7 LIVER 10

1.8 PANCREAS 13

1.9 FREE RADICALS 14

1.10 CONCEPT OF OXIDATIVE STRESS 15

1.11 STREPTOZOTOCIN 17

CHAPTER TWO 23

MATERIALS AND METHODS 23

2.1 MATERIALS 23

2.2 CHEMICALS AND REAGENTS 23

2.3 METHODS: 24

2.4 STATISTICAL ANALYSIS 30
CHAPTER THREE 31
RESULT 31
3.1 FASTING BLOOD GLUCOSE 31
3.2 BODY WEIGHT 32
3.3 REDUCED GLUTATHIONE CONCENTRATION 33
CHAPTER FOUR 34
DISCUSSION AND CONCLUSION 34
4.1 DISCUSSION 34
REFERENCES 38
APPENDIX 42

ABSTRACT

The present study was undertaken to investigate the effect of aqueous extract of *Cymbopogon citratus* on blood glucose, body weight and liver, kidney and pancreas reduced glutathione levels on normal and streptozotocin-induced diabetic rats. Diabetes mellitus was induced in the animals (diabetic control and diabetic treated), by intraperitoneal injections of streptozotocin (45mg/body weight), while the control groups received equal volume of the citrate buffer (pH 4.5) solution intraperitoneally. Streptozotocin treatment significantly increased ($p < 0.05$) blood glucose concentration in the diabetic rats compared to the normal rats. The normal treated and diabetic treated rats were given *Cymbopogon citratus* extract for 21 days (400mg/body weight). The pancreas, livers, and kidneys of the rats were excised and biochemical assay of reduced glutathione was determined. There was a significant ($p < 0.05$) decrease in the fasting blood glucose levels of the normal treated rats when compared with the normal control rats at the end of the 21 days treatment period. Levels of blood glucose in the diabetic rats were significantly increased ($p<0.05$) compared to the normal control rats. However, levels of blood glucose in the diabetic treated rats were not significantly different ($p>0.05$) when compared to the diabetic control rats. There was a significant decrease ($p<0.05$) in body weight in the diabetic rats when compared to the normal control rats. There was no significant % weight ($p>0.05$) gain in the diabetic treated rats when compared to the diabetic control rats and also there was a non-significant ($p>0.05$) decrease in weight in the normal treated rats when compared to the normal control rats. In the liver and the pancreas, the results for reduced glutathione concentration showed that there was no significant difference ($p > 0.05$) in the normal treated rats when compared to the normal control rats, in the diabetic control rats when compared to the normal control rats and in the diabetic treated rats when compared to the diabetic control rats. In the kidney, there was no significant difference observed ($p>0.05$) when the normal treated rats were compared with the normal control rats and when the diabetic treated rats were compared with the normal control rats. However, when the diabetic treated rats were compared with the diabetic control rats, there was a significant difference ($p<0.05$). *Cymbopogon citratus* does have some hypoglycemic and antioxidant properties but further research is needed to ascertain these claims.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Diabetes mellitus is a metabolic derangement associated with sustained hyperglycemia due to the defective insulin secretion and insulin action, resulting to changes in the generation of energy (Chamberlain *et al.*, 2016). Type 1 and type 2 diabetes are the major classes of diabetes mellitus with type 2 diabetes mellitus being the most prevalent of all diabetes cases (Feranimi and Maria, 2014). Type 2 diabetes mellitus is an emerging intercontinental health problem, closely associated to the epidemic of obesity. Recent studies have shown that diabetes mellitus has affected over 500 million people globally in 2018, and this data is likely to double by the year 2045 (Kaiser *et al.*, 2018). Individuals with Type 2 diabetes mellitus are at risk for both microvascular complications and macro vascular complications, owing to hyperglycaemia and individual components of insulin resistance (metabolic syndrome) (Defronzo *et al.*, 2015). Type 2 diabetes is marked with insulin resistance and partially dysfunctional pancreatic β -cells to adequately secrete insulin in response to hyperglycaemia (Crowe and Francis, 2013). Environmental factors (for example, obesity, an unhealthy diet and physical activity) and genetic factors contribute to the multiple pathophysiological abnormalities that are responsible for the impaired glucose homeostasis in Type 2 diabetes mellitus. Insulin resistance and impaired insulin secretion remain the core defects in Type 2 diabetes mellitus, but at least six other pathophysiological abnormalities contribute to the dysregulation of glucose metabolism (Defronzo *et al.*, 2015). The most substantial and safest drugs since sundry times are medicinal plants and they play remarkable role in public and primary healthcare (Oladeji *et al.*, 2019).

Medicinal herbs are therapeutic agents' indispensable in the primary health care system in maintaining exceptional wellbeing and health condition (Bensaba *et al.*, 2015). The untested and unscientific ethnopharmacological knowledge of medicinal plants could well be traced back to the stone age (Bello *et al.*, 2019). Recently, unanticipated advancement has been observed in the use of herbs in primary healthcare system both in Africa and Asia continent. There have been over two third of the world population depending on medical plants as therapeutic drugs; this upsurge could be due to the acceptability, compatibility, adaptability and the little or no side effects of this natural drugs on the human body (Ansioli *et al.,* 2017). Many indigenous medicinal plants have been found to effectively manage diabetes. One of the great advantages of medicinal plants is that they are readily available and have low side effects (Abbas *et al.,* 2017). Available reports show that there are more than 800 plants species that have antidiabetic properties (Rother, 2007). There has been increasing demand for the use of plant products with antidiabetic activity due to some reasons which include; low cost, easy availability and lesser side effects. Thus, plants are being continuously explored for their possible effect as hypoglycemic agents (Eledrisi *et al., 2006).* In Africa, some of the plants commonly used for the treatment of diabetes include, *Bridelia ferruginea,* and *Vernonia amygdalina.* There is experimental evidence for the hypoglycemic effects of some medicinal plants, at least in experimental model of diabetes (Ugadu *et al.,* 2018). In many countries it is traditional to use plants to control Diabetes (Ugadu *et al.,* 2018). Many plants have been used traditionally in Ayurveda and reported to have good antidiabetic activity (Chang *et al.*, 2013).

Cymbopogon citratus, (Lemon grass) is a widely used herb in tropical countries, especially in Southeast Asia (Ugadu *et al.,* 2018). The essential oil of the plant is used in aromatherapy, skin rashes etc. The compounds identified in *Cymbopogon citratus* are mainly

terpenes, alcohols, ketones, aldehyde and esters (Gupta and De, 2012). Studies have revealed that the chemical composition of the essential oil and extracts of cymbopogon citratus varies according to the geographical origin, age, and the nature of the plant (Lawal *et al.*, 2017). The extract from the leaves of the *Cymbopogon citratus* plant are more potent than the root and stems of the plant in the treatment of digestive tracts spasm, high blood pressure, common cold and exhaustion (Olayemi, 2017).

1.2 AIMS AND OBJECTIVES

AIMS

The available drugs (SC insulin, glimepiride, vildagliptin, metformin) used for the treatment of diabetes mellitus is too expensive for people to afford. Hence, the aim of this study is to evaluate the potential of *Cymbopogon citratus* in the treatment of diabetes mellitus in Wistar rats.

OBJECTIVES

1. To determine the effect of *Cymbopogon citratus* on glucose levels in normal and diabetic rats

2. To determine the effect of *Cymbopogon citratus* on body weight in normal and diabetic rats.

3. To determine the effect of *Cymbopogon citratus* on kidney, liver and pancreas oxidative status by estimating reduced glutathione concentration.

1.3 LITERATURE REVIEW

FIGURE 1: LEMON GRASS

1.4 TAXONOMY

- **Domain: Eucaryota**
- **Kingdom: Plantae**
- **Phylum: Spermatophyta**

- **Subphylum: Angiospermae**
- **Class: Monocotyledonae**
- **Order: Cyperales**
- **Family: Poaceae**
- **Genus: Cymbopogon**
- **Specie: Cymbopogon *citratus***

1.5 DIABETES MELLITUS

Diabetes mellitus is a chronic illness characterized by elevated levels of blood glucose, accompanied by disturbed metabolism of fats and proteins. Blood glucose rises because it cannot be metabolized in the cells, due to lack of insulin production by the pancreas or the inability of the cells to effectively use the insulin that is being produced (Gojka, 2016).

TYPES OF DIABETES

TYPE 1 DIABETES MELLITUS

Type 1 diabetes mellitus, also known as autoimmune diabetes is a chronic disease, characterized by insulin deficiency due to pancreatic β-cell damage and leads to hyperglycaemia. Although the age of symptomatic onset is usually during childhood or adolescence, symptoms can sometimes develop sometimes, much later. Although, the aetiology of Type 1 diabetes mellitus is not completely understood, the pathogenesis of the disease is thought to involve T-cell mediated destruction of β -cells. (Katsarou *et al*., 2017).

TYPE 2 DIABETES MELLITUS

Type 2 diabetes mellitus is characterized by insulin resistance and the impaired secretion of insulin and this remain the major defects in type 2 diabetes mellitus. Other pathophysiological defects also play a role in the dysregulation of glucose metabolism. Environmental factors such as obesity, unhealthy diet and physical inactivity contribute to the pathophysiological disturbances that are responsible for the disturbance in glucose homeostasis apart from genetic factors. (Defronzo *et al.,* 2015).

METABOLIC CHANGES ASSOCIATED WITH DIABETES MELLITUS

The metabolic effects of diabetes mellitus results when cells are unable to acquire glucose efficiently from the blood. Instead, the liver, muscles and adipose tissue act as if the body was undergoing starvation despite increased food consumption. Since the body is unable to detect the presence of glucose in the blood, the rate of gluconeogenesis is increased, causing rapid breakdown of proteins and lipids. Lipolysis produces free fatty acids which are substrates for Ketogenesis in the liver (Riserus and Willet, 2009). Hyperglyceamia results partly as a result of the failure of cells to take up and utilize glucose and partly because there is increased gluconeogenesis in the liver fatty acids because the cycle intermediates have been depleted. Acetyl-coA accumulates and this accelerates the formation of the ketones bodies beyond the oxidative capacity of extraheptatic tissues (ketosis). The ketosis in uncontrolled diabetes mellitus may lead to ketoacidosis (the blood pH is decreased) since the ketones bodies produced (acetoacetate and 3-hydroxybutyrate) are relatively strong acids (Malik *et al.,* 2010).

SYMPTOMS OF DIABETES MELLITUS

Symptoms may develop rapidly it may take weeks or months. In type 1 diabetes they usually develop much slowly and may be subtle or absent in type 2 diabetes. The following are symptoms associated with diabetes. (Riserus and Willet, 2009).

GLUCOSURIA

When blood glucose rises from above a certain level it is removed from the body as urine. The maximum blood glucose level reached before sugar spills out is called kidney threshold (usually about 180mg/ml).

POLYURIA

This is frequent and excessive urination. Glucose cannot be passed out of the body alone. Sugars suck up water so that it can flow from the body. This results in polyuria (excessive urination).

POLYDIPSIA

This is the condition known as excessive thirst for water. Loss of water through urination triggers the brain to send a message of thirst. This results in a condition called polydipsia.

POLYPHAGIA

When there is no insulin to aid the entry into the body's cells or when the insulin is not effective to transport glucose through the receptors, the cells starve. This triggers the brain to send a message of hunger, resulting in polyphagia that is excessive hunger.

KETONURIA

The breakdown of fat cells forms fatty acid which passes through the liver to form ketones. Ketones are excreted in the urine and this condition is known as ketonuria that is ketones in the urine.

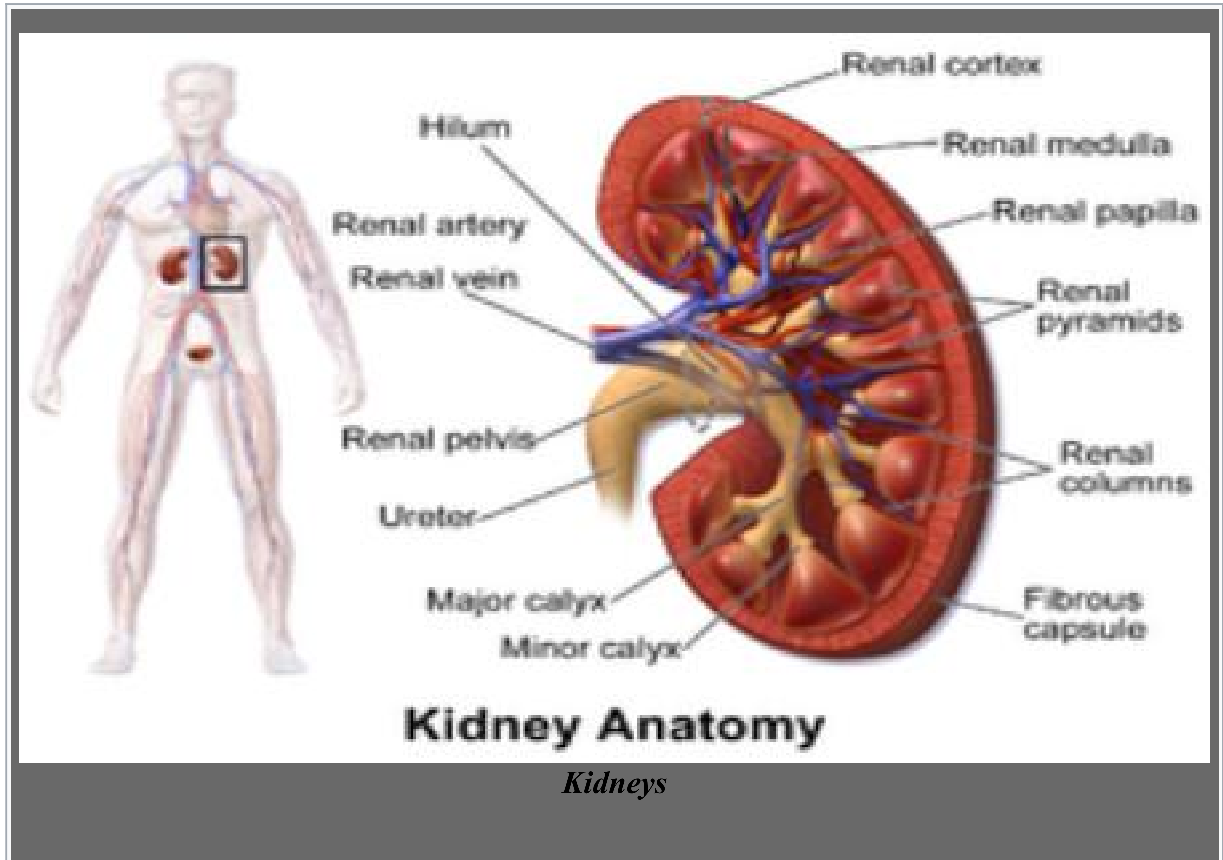
BLURRY SIGHT

Diabetes is known to cause vision loss. Fluctuations in the amount of the glucose and water in the eye lenses during dehydration period can cause blurry eye sight.

FATIGUE AND WEIGHT LOSS

The sugar in diabetics is not able to be metabolized. Since glucose is passed out from the cell, the cell cannot produce the necessary energy needed. In low insulin, glucose hardly enters the cells and the body breaks down the fuels which results in rapid weight loss. Excess sugar suppresses the natural defense mechanism and sugar is excellent for bacterial growth.

1.6 THE KIDNEYS



Kidneys

Figure 2: Structure of the kidney

Source: https://www.wikiwand.com/en/Renal_medulla

The kidneys are two bean-shaped organs found in vertebrates. They are located on the left and right in the retroperitoneal space, and in adult humans are about 12 centimetres (4 ½ inches) in length (Christopher, 2012). They receive blood from the paired renal arteries; blood exits into the paired renal veins. Each kidney is attached to a ureter, a tube that carries excreted urine to the bladder.

The nephron is the structural and functional unit of the kidney. Each human adult kidney contains around 1 million nephrons, while a mouse kidney contains only about 12,500 nephrons. The kidney participates in the control of the volume of various body fluids, fluid osmolality, acid-base balance, various electrolyte concentrations, and removal of toxins. Filtration occurs in the glomerulus: one-fifth of the blood volume that enters the kidneys is filtered.

1.7 LIVER

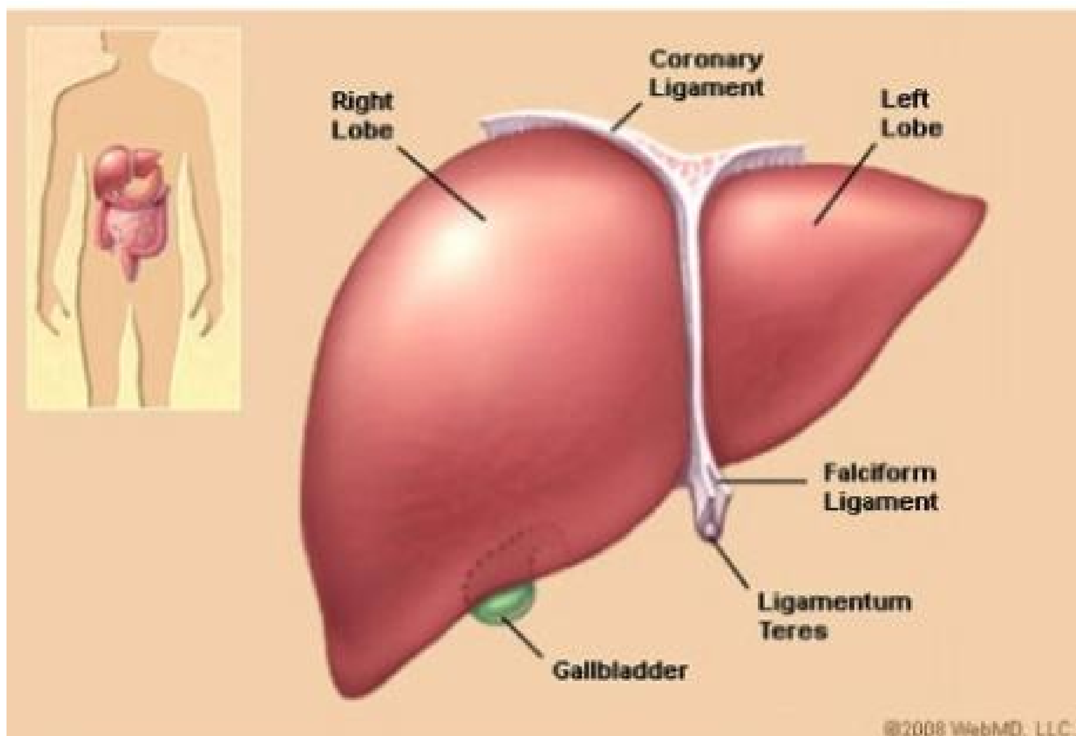


Figure 3: Structure of the liver

Source: https://quizlet.com/8624968/digestive-system-ii-flash-cards/

liver is the largest solid organ in the body. The liver is a vital organ that helps to regulate many crucial metabolic functions and is responsible for the body homeostasis maintenance (Mayuren

et al., 2010) It removes toxins from the body's blood supply, maintains healthy blood sugar levels, regulates blood clotting, and performs hundreds of other vital functions. It is located beneath the rib cage in the right upper abdomen. The basic functional unit of the liver is the liver lobule, which is a cylindrical structure several millimeters in length and 0.8 to 2 millimeters in diameter. The liver is composed of 50,000 to 100, 000 individual lobules (Guyton and Hall, 2004). The liver lobule is constructed around a central vein that empties into the hepatic veins and then into the vena cava.

Functions of the liver

The liver is an essential organ of the body that performs over 500 vital functions. These include removing waste products and foreign substances from the bloodstream, regulating blood sugar levels, and creating essential nutrients. Here are some of its most important functions:

Albumin Production: Albumin is a protein that keeps fluids in the bloodstream from leaking into surrounding tissue. It also carries hormones, vitamins, and enzymes through the body.

Bile Production: Bile is a fluid that is critical to the digestion and absorption of fats in the small intestine. The liver produces and secrete bile that is needed to breakdown and digest fatty acid.

Detoxification: All the blood leaving the stomach and intestines passes through the liver, which removes toxins, byproducts, and other harmful substances. Toxic chemicals of both internal and external origin constantly bombard the liver. Our normal everyday metabolism produces a wide range of toxins that are neutralized by the liver. (NIDDK, 2000).

Regulates Amino Acids: The production of proteins depends on amino acids. The liver makes sure amino acid levels in the bloodstream remain healthy. The liver plays an important role in protein and lipoprotein synthesis and catabolism. (Neera *et al.,* 2015)

Regulates Blood Clotting: Blood clotting coagulants are created using vitamin K, which can only be absorbed with the help of bile, a fluid the liver produces.

Resists Infections: As part of the filtering process, the liver also removes bacteria from the bloodstream.

Stores Vitamins and Minerals: The liver stores significant amounts of vitamins A, D, E, K, and B12, as well as iron and copper.

Processes Glucose: The liver removes excess glucose (sugar) from the bloodstream and stores it as glycogen. As needed, it can convert glycogen back into glucose.

ANATOMY OF THE LIVER

The liver is reddish-brown and shaped approximately like a cone or a wedge, with the small end above the spleen and stomach and the large end above the small intestine. The entire organ is located below the lungs in the right upper abdomen. It weighs between 3 and 3.5 pounds. The liver consists of four lobes: the larger right lobe and left lobe, and the smaller caudate lobe and quadrate lobe. The left and right lobe are divided by the falciform ("sickle-shaped" in Latin) ligament, which connects the liver to the abdominal wall. The liver's lobes can be further divided into eight segments, which are made up of thousands of lobules (small lobes). Each of these lobules has a duct flowing toward the common hepatic duct, which drains bile from the liver.

1.8 PANCREAS

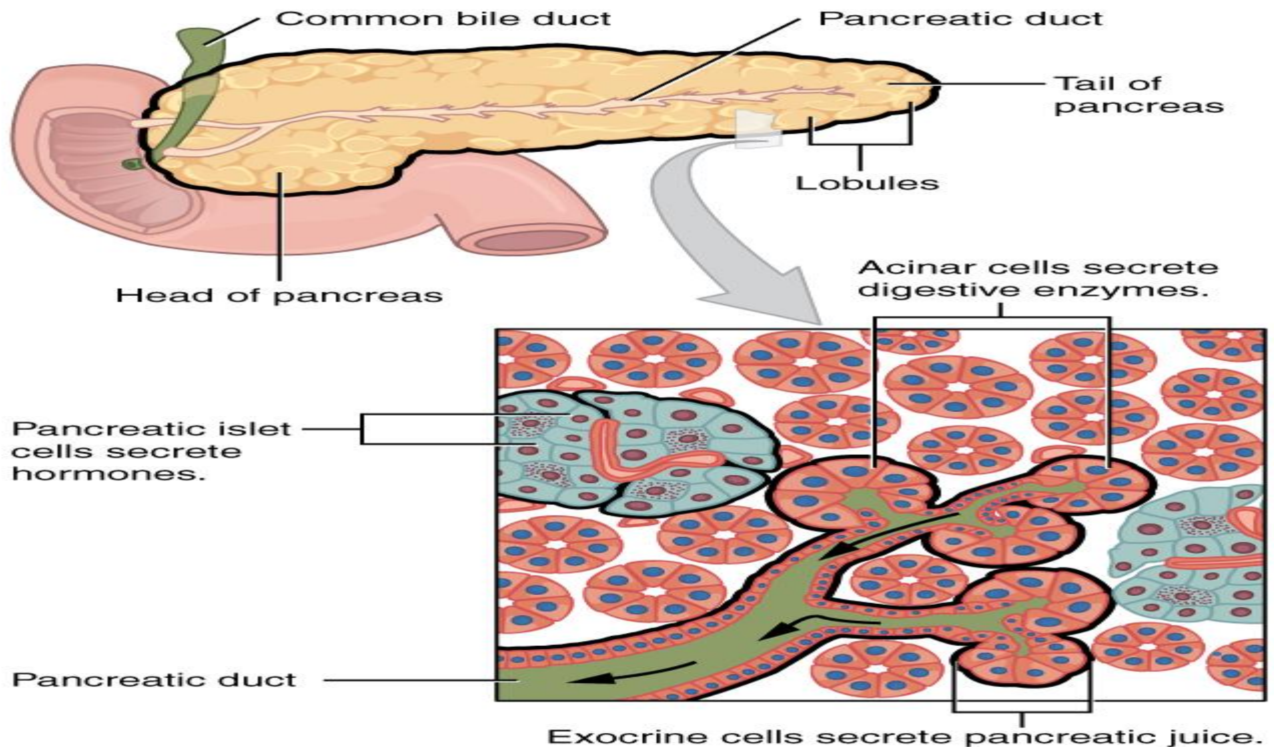


Figure 4: Structure of the pancreas

Source: https://encrypted-tbn0.gstatic.com/images?q=tbn:ANd9GcTpFc47I0T7-xuCdo1uOnRZ978bgySRpdcKfQ&usqp=CAU

The pancreas is a long, flat gland present in the stomach, and divided into head, body and tail. It plays a significant role in the digestive system and in the regulation of blood sugar. The islets of langerhan produces multiple hormones via the endocrine cells (stitzel et al., 2015). Metabolic homeostasis in mammals is tightly regulated by the complementary actions of insulin and glucagon. The secretion of these hormones from pancreatic beta cells and alpha cells, respectively, is controlled by metabolic, endocrine and paracrine regulatory mechanism and is essential essential for the control of blood glucose levels,

1.9 FREE RADICALS

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants (Cheeseman and Slateter, 1999). The most important oxygen-containing free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical. These are highly reactive species, capable in the nucleus, and in the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates and lipids (Young and Woodside, 2001). Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicals include all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets.

Production of free radicals in the human body

Free radicals and other (Reactive Oxygen Species) ROS are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals. Free radical formation occurs continuously in the cells as a consequence of both enzymatic and nonenzymatic reactions. Enzymatic reactions, which serve as source of free radicals, include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis, and in the cytochrome P-450 system (Liu *et al*., 1999). Free radicals can also be formed in nonenzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing reactions.

Some internally generated sources of free radicals are Mitochondria, Xanthine oxidase, Peroxisomes, Inflammation, Phagocytosis, Exercise. Some externally generated sources of free radicals are: Cigarette smoke, Environmental pollutants, Radiation, Certain drugs, pesticides, Industrial solvents, Ozone.

1.10 CONCEPT OF OXIDATIVE STRESS

The term is used to describe the condition of oxidative damage resulting when the critical balance between free radical generation and antioxidant defenses is unfavorable (Rock *et al.,* 1996). Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids (McCord, 2000). Short-term oxidative stress may occur in tissues injured by trauma, infection, heat injury, hypertoxia, toxins, and excessive exercise. These injured tissues produce increased radical generating enzymes (e.g., xanthine oxidase, lipoxygenase, cyclooxygenase) activation of phagocytes, release of free iron, copper ions, or a disruption of the electron transport chains of oxidative phosphorylation, producing excess reactive oxygen species (ROS). The initiation, promotion, and progression of cancer, as well as the side-effects of radiation and chemotherapy, have been linked to the imbalance between ROS and the antioxidant defense system. ROS have been implicated in the induction and complications of diabetes mellitus, age-related eye disease, and neurodegenerative diseases such as Parkinson's disease (Rao *et al.,* 2006).

OXIDATIVE STRESS AND TYPE 2 DIABETICS MELLITUS

Various metabolic processes can lead to oxidative stress. A condition in which oxidation is an important pathogenic link is type 2 diabetes mellitus. insulin resistance is the major constituent

to which a compensatory hypersecretion of insulin is linked in this disease. The inactivation of signaling mechanisms between insulin receptors and the glucose transport mechanisms resulting ultimately to insulin resistance. (Cheng *et al.,* 2018).

Diabetes on its own is also a generator of oxidative stress with atherogenetic consequence. Generation of superoxide ions in the endothelia cells at the mitochondrial level can be induced by hyperglycemia. In diabetic condition, electron transfer and oxidative phosphorylation are decoupled leading to superoxide anions production and insufficient ATP synthesis. So, the therapeutic strategy in diabetes management is the prevention of the damage caused by oxidation. Accumulation of free fatty acids with a consecutive increased accumulation of intramyocellular lipids were thought to be the cause of insulin resistance and the cell death of beta-pancreatic cells. Recent study show that free radicals and glucose can initiate free radical formation through mitochondrial mechanisms and NADPH oxidase in adipocytes, beta cells, muscle cells and other cells types. free fatty acids penetrate cellular organs, including the mitochondria where high levels of reactive oxygen species can cause peroxidation and damage.

Studies carried out recently showed that insulin resistance and type 2 diabetes mellitus are closely linked with a decrease in mitochondrial oxidative function in the skeletal muscle. In this type 2 diabetes mellitus, the mitochondria become smaller, rounder and prone to producing superoxide. Disorders of the mitochondrial electron transport chain, excessive generation of reactive species and lipoperoxides, as well as decrease in antioxidant mechanisms have been observed in type 2 diabetes mellitus and obesity. (Sharifi-Rad *et al*., 2020).

1.11 STREPTOZOTOCIN

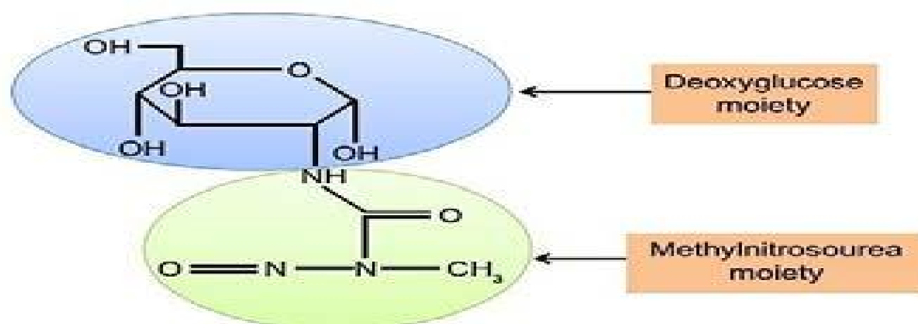


Figure 5: chemical structure of streptozotocin (Wu *et al.,* 2015).

A chemical that has been successfully been used for inducing diabetes in rats is streptozotocin. (Guo *et al*., 2018). Streptozotocin was for the first time reported to induce diabetes in 1963 and since then, it has been used in several studies for inducing diabetes. (Delmalgahaes *et al*., 2019). Streptozotocin, is a broad spectrum antibiotic produced by the bacterium *Streptomyces achromogens*. It contains a glucose molecule linked to a highly reactive methy-nitrosourea moiety that is thought to exert streptozotocins cytotoxic effects, while the glucose moiety directs the chemical to the pancreatic β cells. (Vavra *et al*., 1959). The selective pancreatic β cell toxicity and diabetic condition initiated by streptozotocin induction is related to the glucose moiety in its chemical structure which allows streptozotocin to enter the β cell via the low affinity glucose-2-transporter in the plasma membrane. (Elsner *et al*., 2007). Due to the fact that β-cells of the pancreas are more active than other cells in taking up glucose and are more sensitive than other cells to streptozotocin challenge. This statement is validated by the observation that insulin producing cells that do not express the GLUT2 glucose transporter are resistant to streptozotocin toxicity and only become vulnerable to the toxicity of this compound

after the expression of the GLUT2 transporter protein in the plasma membrane (Rerup, 1970). Moreover, other cells that express the GLUT2 transporter such as hepatocytes and renal tubular cells are also susceptible to streptozotocin. This explains why experimental animals inducted with streptozotocin, tend to have renal and liver damage (Eleagu *et al*., 2013). In addition, non β-cells such as: α-cells and the extra pancreatic parenchyma remain intact after streptozotocin administration, indicating the β-cells selective properties of streptozotocin (Lenzen, 2007). Streptozotocin also causes cardiac and adipose tissue damage and increases oxidative stress, inflammation and endothelial dysfuction (Valentovic *et al*., 1997).

The half-life of streptozotocin is short due to its rapid metabolism in the liver and excretion by renal excretion (Eleazu *et al*., 2013). When streptozotocin is excreted from the body, further impairment of the fuction of the liver or kidney is attributed to the effects of diabetic hyperglycemia. This is the basis for the studies on the mechanisms of streptozotocin diabetic complications in kidney, pancreas and liver as well as other organs like the brain, heart and muscles (Akinlade *et al.,* 2021).

1.12 BIOCHEMICAL BASES FOR THE CYTOTOXICITY OF STREPTOZOTOCIN

Streptozotocin, a structural analogue of glucose and N-acetylglucosamine is taken up by the pancreatic β-cells via the GLUT2 transporter where it induces the death of β-cells via DNA fragmentation, excerted by the nitrosourea moiety. The three major pathway associated with β-cell death are: DNA methylation, nitric oxide production and the production of free radicals.

METHYLATION OF DNA

DNA meethylating activity of the methyl nitosourea moiety of streptozotocin leading to the damaging of the DNA with necrosis of the pancreatic β-cells, through the depletion of cellular energy stores (Mulan *et al*.,1999). The activation of poly ADP-ribose polymerase, (PARP) in an attempt to repair the damaged DNA results in the cellular depletion of NAD^+ and ATP stores as a result of overstimulation of DNA repair mechanism (Piepper *et al*., 1999). Also, streptozotocin can also react at other DNA sites such as the ring nitrogen and the exocyclic oxygen atoms of DNA bases, predominantly producing 7-methylguanine, 3-methyladenine which leads to DNA breaks, activates PARP and subsequently depletes NAD^+ (Stauffacher *et al.,* 1970).

NITRIC OXIDE PRODUCTION

Another mechanism in which streptozotocin induces diabetonic effect that results in cell death has been attributed to its ability to act as nitric oxide donor in pancreatic cells which leads to the inhibition of the activity of aconitase, resulting to the akylation and damage of DNA (Soms *et al.,* 2000). Streptozotocin increases the activity of guanyl cyclase and the formation cyclic guanosine monophosphate (cGMP), which are characteristics action of nitric oxide. β-cells are particularly sensitive to the damaging effect of nitric oxide and free radicals because of its low levels of free radicals scavenging enzyme (Spinas, 1999).

REACTIVE OXYGEN SPECIES

Oxidative stress, is as a result of an imbalance between free radical production and antioxidant defenses. (McCord, 2000). Oxidative stress has recently been shown to be

responsible at least partially for the dysfunction of β-cell attributed to glucose toxicity in hyperglycemia. Several reaction mechanisms are thought to be involved in the genesis of oxidative stress in both diabetic patients and diabetic animals and they include: protein glycation, formation of advanced glycation products and the polyol pathway (West, 2000). During these biochemical processes, reactive oxygen species are produced and causes damage to the tissue (Matsuoka *et al.,* 1998). Streptozotocin treatment induces a significant change in malonaldehyde but decreases antioxidant enzymes such as: catalase, glutathione peroxidase and superoxide dismutase activities when compared to the control animals in experiment (Gul *et al*., 2002). A reduction in the antioxidant enzyme activity and increase in malonaldehyde activity is an indication of the susceptibility of pancreas to streptozotocin induction of oxidative stress (Henrikson, 2002).

An important contribution of reactive oxygen species during streptozotocin metabolism is the production of uric acid as the ATP degradation by xanthine oxidase from hypoxanthine. This reaction produces reactive oxygen species such as superoxide and hydroxyl radicals emanating from hydrogen peroxide dismutation during hypoxanthine metabolism coupled with the fact that the cells of the pancreas is devoid of catalase and glutathione peroxidase, accelerates the process of β-cell death. The free radicals such as O^{2-} and OH^- produced from hydrogen peroxide eventually generates free radicals which can cause lipid peroxidation, resulting in the formation of hydroperoxy fatty acids and endoperoxides that increases the formation of malonaldehyde. This increase in the reactive oxygen species production has also been reported to inhibit aconitase which protects mitochondria DNA from degradation. (Vergani *et al.,* 2004).

Glutathione systems

The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases, and glutathione S-transferases. This system is found in animals, plants, and microorganisms (Meister, 1983). Glutathione peroxidase is an enzyme containing four selenium-cofactors that catalyze the breakdown of hydrogen peroxide and organic hydroperoxides. There are at least four different glutathione peroxidase isozymes in animals (Brigelius, 1992). Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides. The glutathione S-transferases show high activity with lipid peroxides. These enzymes are at particularly high levels in the liver and also serve in detoxification metabolism. Ascorbic acid or "vitamin C" is a monosaccharide antioxidant found in both animals and plants. As it cannot be synthesized in humans and must be obtained from the diet, it is a vitamin (Smirnoff, 2001). Most other animals are able to produce this compound in their bodies and do not require it in their diets. In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins. Ascorbic acid is a reducing agent and can reduce and thereby neutralize ROS such as hydrogen peroxide (Padayatty *et al*., 2003) In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the antioxidant enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants (Shigeoka *et al*., 2002).

Glutathione

Glutathione is a cysteine-containing peptide found in most forms of aerobic life (Meister, 1988). It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the

reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems as well as reacting directly with oxidants ((Meister, 1988). Due to its high concentration and central role in maintaining the cell's redox state, glutathione is one of the most, important cellular antioxidants (Matil, 1999). In some organisms, glutathione is replaced by other thiols, such as by mycothiol in the actinomycetes, or by trypanothione in the kinetoplastids (Fairlamb and Cerami, 1992).

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

Plant Material

Fresh leaves *Cymbopogon citratus* were obtained from Uselu market, Benin City, Edo State. Nigeria. The plant was identified by a plant taxonomist at the Department of Plant Biology and Biotechnology, University of Benin Nigeria and was given a voucher number UBH-C451.

ANIMALS

Forty-five white (45) Wistar rats were bought from the animal house unit of Biochemistry Department, University of Benin, Benin City.

ANIMAL FEED

Pellet feeds were gotten from Chikun feeds in Adolor, Benin city. (Crown Mills limited, Lagos, Nigeria).

2.2 CHEMICALS AND REAGENTS

Analytically graded chemicals were used for all the experiments.

Streptozotocin (Sigma Aldrich, London).

Chloroform (Sigma-JHD, Germany).

Picric Acid (Dow, United State).

Distilled water

Citric Acid (May and Baker, England).

Trichloroacetic acid

Sodium citrate (May and Baker, England).

5.5'-dithiobis-2-nitrobenzoic acid (DTNB)

Phosphate buffer

Apparatus/Equipment

Apparatus and Equipment used for this study and their sources include:

Atomic Absorbance Spectrophotometer (Buck Scientific, USA), Centrifuge (Thermo scientific, UK), Weighing balance1 (Gallenkamp, England), weighing balance 2 (Atom-A110C, China), Micropipette (Mettler Delta Range, Switzerland). PH Meter (PHep, USA), Centrifuge tube, Capillary Tubes, Fine Dissecting Needle (Pyrex, England), dissecting set (Gold Cross, England). Glucometer (Yasee, China), scissors, Glucometer Strips (Yasee, China), Syringes, Hand gloves, Nose mask, Pin, Gavage, Cages, Ceramic Mortal crucibles, Plates and Pestle, Filter paper, Funnel, 500ml Conical flask (Technico, India), Beakers (Pyrex, England),

2.3 METHODS:

Preparation of *Cymbopogon citratus* extract

The *Cymbopogon citratus* leaves were rinsed with water and air dried at for 7 days and were milled thoroughly into powdery form using mechanical blender. The samples were weighed using weighing scale into 50grams each and mixed with 1 liter of distilled water and stirred. The mixture was stirred and brought to boil. After 10minutes of boiling, the mixture was allowed to cool and decanted into a container lined with a muslin cloth and then evaporated to dryness using

a rotary evaporator at $60^{o}C$ in the Department of Pharmacy, Faculty of Pharmacy, University of Benin, Benin City. Each batch of dried extract and container was weighed separately and collectively to determine extract weight and stored at $4^{0}C$.

Experimental Animals and Research Design

Forty-five (45) albino rats of Wistar strain weighing between 80g- 120g were used for this study. They were housed in the animal house of Biochemistry Department, University of Benin, Benin City. The animals were grouped into four (4) as follows: normal control, normal treated, diabetic control and diabetic treated. The wistar rats under normal control are 10 in number, the normal treated rats are 5 in number, the diabetic treated and diabetic control rats are 15 each. They were all fed with rat dry pellet diet and water *ad libitum,* maintained under standard laboratory conditions with dark and light cycle (12:12 hour). The animals were acclimatized for 7days before commencement of this work.

Preparation of streptozotocin

Streptozotocin was stored at a very low temperature of $-4^{o}C$ until it was time to be administered. Within a 6 minutes' interval 45mg streptozotocin was dissolved in 1ml (0.1M) citrate buffer (P^{H} 4.5). (Sadique *et al*, 1987).

ADMINISTRATION OF STREPTOZOTOCIN

The animals were given 10% sucrose water 6 hours prior to the administration of streptozotocin to prevent hypoglycemia and were fasted overnight. Each animal in the diabetic groups were given a single injection containing the freshly prepared streptozotocin and citrate buffer solution (45mg/kg body weight) intraperitoneally while the control rats were given the buffer only

(1ml/kg body weight). The blood glucose levels were determined before administration, and taken again after 48hours.

ADMINISTRATION OF EXTRACT

The treated groups were given *Cymbopogon citratus* extract at 400mg/kg body weight. The extract was administered orally using a gavage every day for 3 weeks.

PREPARATION OF SAMPLES AND GLUCOSE EVALUATION

At the end of the treatment period, the animals were anaesthetized with chloroform vapor, quickly brought out of the jar and sacrificed. Five milliliter (5ml) of the blood was collected by cardiac puncture into plain bottles. The serum was used to assay for blood glucose level.

PREPARATION OF TISSUE HOMOGENATES (LIVER, KIDNEY AND PANCREAS).

The excised organ (1g of liver, kidney or pancreas), was weighed and then homogenized in normal saline (0.9% w/v). The homogenate was centrifuged at 3000rpm for 15minutes after which the clear supernatant was collected into plain bottles and stored at -20°C until it was required for biochemical assay.

DETERMINATION OF BODY WEIGHT

Body weight was determined using the method of Tonyushkina and Nichols, (1983) as described in the weighing balance manual.

PRINCIPLE

When an object is placed on a digital weighing balance, its weight causes its internal strain gauge to deform. The weighing balance converts that amount of deformation to an electrical signal, runs the signal through a digital converter, and shows the weight on the balance display.

PROCEDURE

The body weights for each animal was recorded using a weighing balance. Their initial weights (immediately after purchase) and final weights (a day before sacrifice) were recorded.

DETERMINATION OF FASTING BLOOD GLUCOSE

Fasting blood glucose was determined using the method of Barham and Trinder, (1972) as described in the glucometer manual.

PRINCIPLE

The enzyme portion of the glucose meter is generally packaged in a dehydrated state in dehydrated state in a disposable strip or reaction cuvette. Glucose in the patients' blood sample rehydrates and reacts with the enzymes to produce a product that can be detected. Most meters generate hydrogen peroxide or an intermediary that can react with a dye, resulting in a colour change proportional to the concentration of glucose in solution.

PROCEDURE

Fasting blood glucose was taken using YASEE glucometer strips. The rats were fasted for 12-13 hours from 6pm to 7am, the blood samples were taken by sniping the tip of the tail of the rats with blood collection needle, then a test strip was removed from the vial and inserted into the glucometer. Blood drops at the tip of the rats' tail was placed in the glucose strips. The blood

glucose result was shown on the meter display screen. The tail of the rats were cleaned with cotton wool soaked in methylated spirits after. Each animals blood glucose level was first recorded at base, then on day 1, day 7, day 14 and day 21 after the administration of streptozotocin.

DETERMINATION OF REDUCED GLUTATHIONE CONCENTRATION IN LIVER, KIDNEY AND PANCREAS.

Reduced glutathione concentration was determined using the method described by Ellman, (1959).

PRINCIPLE

Ellman method is a kinectic method based on the reduction of 5, 5^1-dithiobis-2-nitrobenzoic acid (DTNB) to trinitrobenzene by glutathione. The optical density of this reduced disulphide compound absorbance at 412nm can be measured by spectrophotometry.

Procedure

To 1.0 mL of plasma, 2.5 mL of 10 % TCA was added and centrifuged at 3000 g for 10 min. Then, 1.0 mL of the supernatant was treated with 0.5 mL of Ellman's reagent (0.0189 % DTNB and 1 % sodium citrate) and 3.0 mL of 0.3 M phosphate buffer (pH 8.0). The yellow colour developed was read immediately at 412 nm and expressed as µM GSH plasma.

Calculation

$$\text{Concentration of GSH} = \frac{A_{test} \times \text{Conc. of Standard}}{}$$

$A_{standard}$

$$\% \text{ Glutathione Reduced} = \frac{(A_0 - A_1) \times 100}{A_0}$$

where A_0 = Absorbance of reference sample

A_1 = Absorbance of sample

Redox Status = GSH/GSSG µM GSH

2.4 STATISTICAL ANALYSIS

The results were expressed as as mean ± standard error of mean (S.E.M). The data were statistically analysed using student's t-test with the program statistical package for social sciences (S.P.S.S) Version 17. The data means were considered different at $p \leq 0.05$.

CHAPTER THREE

RESULT

3.1 FASTING BLOOD GLUCOSE

There was a non-significant ($p>0.05$) decrease in the fasting blood glucose levels of the normal treated rats when compared with the normal control rats during the 21 days' treatment period. However, there was a significant ($p<0.05$) increase in the fasting blood glucose levels of the Diabetic treated rats when compared with the Diabetic control rats.

Table 3.1: Fasting blood glucose of rats for the 21 days' treatment period

GROUPS	**Basal Glucose (mg/dl)**	**Day 1 mg/dl)**	**Day 7 mg/dl)**	**Day 14 mg/dl)**	**Day 21 mg/dl)**
Normal Control (n=5)	93.25 ± 12.22^{a}	90.50 ± 8.70^{a}	75.75 ± 12.58^{a}	85.00 ± 15.86^{a}	91.25 ± 4.39^{a}
Normal treated (n=5)	79.50 ± 11.11^{a}	62.50 ± 9.40^{a}	61.50 ± 5.90^{a}	54.75 ± 4.48^{a}	54.75 ± 6.07^{b}
Diabetic Control (n=5)	69.75 ± 8.82^{c}	125.75 ± 26.99^{c}	97.00 ± 7.95^{c}	166.50 ± 64.74^{c}	94.00 ± 13.95^{c}
Diabetic Treated (n=5)	45.00 ± 4.24^{d}	230.50 ± 82.58^{c}	193.75 ± 37.71^{d}	183.50 ± 53.15^{c}	207.75 ± 54.12^{d}

Values are represented as mean ± SEM for each of the four groups. Values with the same superscripts are not significantly different ($p>0.05$) while values with different superscript are significantly different from each other ($p<0.05$).

3.2 BODY WEIGHT

There was a significant % weight gain in the normal control rats but a slight weight gain in the Diabetic control rats. But when compared respectively with the normal treated and diabetic treated group, there was a slight % weight gain.

Table 3.2: The initial, final and percentage change in weight of the corresponding groups within the 21 days treatment period.

Groups	**Initial weight**	**Final weight**	**% weight gain or loss**
Normal Control	$119.01 \pm 4.32639^{a,b}$	185.50 ± 5.68^{a}	+55.87%
Normal Treated	128.00 ± 2.24^{b}	185.25 ± 9.01^{a}	+44.72%
Diabetic control	$147.79 \pm 0.88^{b,c}$	$154.50 \pm 12.13^{b,c}$	+4.54%
Diabetic Treated	$140.19 \pm 2.48^{b,c}$	$142.75 \pm 10.04^{b,c}$	+1.86%

Values are represented as mean ± SEM for each of the four groups. Values with the same superscripts are not significantly different ($p>0.05$) while values with different superscript are significantly different from each other ($p<0.05$), (+ means weight gain, - means weight loss).

3.3 REDUCED GLUTATHIONE CONCENTRATION

The diabetic treated group was significantly different at $p \leq 0.05$ when compared with normal and diabetic control rats for the Kidney. However, the diabetic treated rats were non-significantly different at $p \geq 0.05$ when compared with normal and diabetic control rats for the Liver and Pancreas.

TABLE 3.3: The effect of Cymbopogon Citratus (LEMON GRASS) extract on reduced glutathione (GSH) levels of the liver, kidney and pancreas of normal and streptozocin-induced diabetic rats.

ORGANS / GROUPS	LIVER (μM GSH)	KIDNEY (μM GSH)	PANCREAS (μM GSH)
NORMAL CONTROL	0.0421 ± 0.0074^{a}	0.0245 ± 0.0022^{a}	0.0710 ± 0.0074^{a}
NORMAL TREATED	$0.0373 \pm 0.0091^{a,c}$	$0.0198 \pm 0.0037^{a,c}$	$0.0494 \pm 0.0094^{a,c}$
DIABETIC CONTROL	$0.0474 \pm 0.0173^{a,c}$	$0.0294 \pm 0.0038^{a,c}$	$0.0737 \pm 0.0094^{a,c}$
DIABETIC TREATED	$0.0407\pm 0.0023^{a,c}$	$0.1025 \pm 0.0081^{b,d}$	$0.0819 \pm 0.0207^{a,c}$

Values are Mean ± SEM. Data with different alphabetical superscript are significantly different at $p \leq 0.05$ when compared with control group. Whereas data with similar alphabetical superscript are not significantly different at $p \geq 0.05$.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

In this study, the results showed the effects of both the *Cymbopogon citratus* extracts on blood glucose, weight and reduced glutathione levels of the liver, kidney and pancreas of streptozotocin-induced diabetic rats. From the results obtained, streptozotocin treatment causes significant increase in blood glucose concentration. Thus, this study indicates the susceptibility of the beta cells of the pancreas to streptozotocin. This observation is in agreement with the studies done by Adewole and Martin-Caxton, (2006). After days 14 and 21, normal treated (NT) rats compared to normal control(NC) rats had no significant difference in their blood glucose levels ($P>0.05$). The diabetic control rats compared to the normal control rats on the other hand had a significant difference when their glucose levels were compared ($P<0.05$). The diabetic treated rats was significantly different at $P<0.05$ when compared to the diabetic control rats. The blood glucose levels of the diabetic treated rats was much higher than that of the diabetic control. Ugadu *et al.,* (2018), reported that the hypoglycemic plant they studied, *Cymbopogon citratus* significantly ($p<0.05$) enhanced the restoration of glucose levels by reversing the alteration caused by streptozotocin but the result observed in this study did not agree with their findings due to the fact that blood glucose levels of the diabetic treated rats was much higher than that of the diabetic control rats.

The percentage weight gain in the normal treated rats were not significantly different (P>0.05), when compared to normal control rats. However, there was a significant difference in percentage weight gain (P<0.05) when the diabetic control rats were compared to the normal control rats; the diabetic control rats showed much less percentage weight increase. This loss in weight in the diabetic rats is due to the fact that streptozotocin induced diabetes is characterized by loss in the weight of the body which is as a result in the degeneration of structural proteins (Eleazu *et al.,* 2013). However, there was no significant (P>0.05) percentage weight gain observed when the diabetic treated rats were compared to the diabetic control rats.

In the liver, the results for reduced glutathione was not significantly different (P>0.05) when the normal treated (NT) rats were compared to the normal control (NC) rats. There was also no significant difference at P>0.05 when the diabetic control (DC) rats were compared to the normal control (NC) rats and when the diabetic treated (DT) rats were compared to the diabetic control (DC) rats.

Pancreatic results for reduced glutathione concentrations showed that there was no significant difference (P>0.05), when the normal treated (NT) rats were compared to the normal control (NC) rats. There was also no significant difference at P>0.05 when the diabetic control (DC) rats were compared to the normal control (NC) rats and the same results were also seen when the diabetic treated (DT) rats were compared to the diabetic control (DC) rats; there was no significant difference at P>0.05. Panda and Kar, (1998) reported significantly increased activity of some antioxidant enzymes in liver i.e., superoxide dismutase, catalase and glutathione following treatment with aqueous extract of *Ocimum sanctum*. The result observed in this study did not agree with their findings due to the fact that there was no significant difference (P>0.05) in reduced glutathione levels when the normal treated rats were compared to the diabetic control

rats, diabetic control rats were compared to the normal control and when the normal treated were compared to the normal control in the liver and pancreas of the rats.

In the kidney, there was no significant difference ($P>0.05$) when the normal treated (NT) rats were compared to the normal control (NC) rats and when the diabetic treated (DT) rats were compared to the normal control (NC) rats. However, when diabetic treated rats were compared to the diabetic control rats, there was a significant difference ($P<0.05$). This result correlates with the result of Ademuyiwa and Grace, 2015. It can still be suggested that the medicinal plant, at some point, enhanced the oxidative status of test animals. Chronic oxidative stress due to hyperglycemia may play an important role in progressive β-cell dysfunction (Tiwari and Rao, 2002), since pancreatic islets have low expression of antioxidant enzymes (Tiedge *et al*., 1997). The significant increase in kidney glutathione activities seen in this study is particularly advantageous as it may play a role in any oxidant related events which contribute to the tubule cell injury which occurs during acute renal failure (Weinberg, 1992).

CONCLUSION

Results from this study revealed that streptozotocin causes significant increase in blood glucose concentration and *Cymbopogon citratus* does have some antioxidant properties as seen in the increase in reduced glutathione levels in the kidney of the diabetic treated rats when compared to the diabetic control. This study can be utilized as a basis for the formulation and enhancement of additional research, however further research is required to support the use of *Cymbopogon citratus* as a remedy for diabetes, since the hypoglycaemic effect was only significant ($p<0.05$) in the normal treated rats.

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APPENDIX

APPENDIX

WEIGHT OF RATS

Table 1 showing weight of rats used for this study

Normal control group

Name	Initial weight (g)	Final weight (g)
H/B	114.2	198
T	131.9	174
B	113.8	192
H/T	116.15	178
H	132.9	203

Diabetic control group

Names	Initial weight(g)	Final weight(g)
	cage 1	
H/T	142.4	178
H/B	135.58	174
A	139.5	192
A/RL	146.65	136
2L	147.11	163
T/2L	147	134
H/2L	150.4	185
	cage 2	
H/T	134.58	170
H	140.41	134
B	146.62	144
H/A	139.13	123

B/T	133	167
H/RL	132.5	133

Normal treated group

Name	Initial weight(g)	Final weight(g)
H/B	133.01	166
H/RL	128.61	203
H/T	122.11	198
B	128.28	174
A	120.55	185

Diabetic treated group

Name	Initial weight(g)	Final weight(g)
	cage 1	
A	137.92	98
B/RL	155.16	144
T/2L	149.4	148
2L	140.05	166
H/B/RL	140.1	185
	cage 2	
H/2L	136.1	141
H/A	159	140
H	155	200
RL	144.7	179
T	158.3	199
A	162.9	132

GLUCOSE LEVELS OF RATS

Table showing the various glucose levels of rats used for the study

Normal control

Name	Basal glucose mg/dl	Day 1 mg/dl	Day 7 mg/dl	Day 14 mg/dl	Day 21 mg/dl
H/B	116	100	111	108	85
T	78	79	75	116	86
B	112	110	64	52	104
H/T	67	73	53	64	90
H	84	85	60	62	94

Normal treated

Name	Basal glucose mg/dl	Day1 mg/dl	Day 7 mg/dl	Day 14 mg/dl	Day 21 mg/dl
H/B	48	85	59	67	61
H/RL	83	39	59	46	39
H/T	87	63	50	51	52
B	100	63	78	55	67
A	102	59	61	68	53

Diabetic control

Name	Basal glucose mg/dl	Day 1 mg/dl	Day 7 mg/dl	Day14 mg/dl	Day 21 mg/dl
		Cage 1			
H/T	46	73	80	111	73
H/B	68	191	88	73	77
A	87	91	115	124	92
A/RL	78	148	105	358	134
2L	67	157	111	346	122
T/2L	61	156	148	442	192
H/2L	61	132	80	96	107
		Cage 2			
H/T	46	76	58	92	88

H	62	151	401	192	324
B	56	92	248	438	535
H/A	74	211	251	398	370
B/T	62	101	68	54	91
H/RL	75	111	271	104	320

Diabetic treated

Name	Basal glucose mg/dl	Day 1 mg/dl	Day 7 mg/dl	Day 14 mg/dl	Day 21 mg/dl
		Cage 1			
A	45	464	160	128	315
B/RL	33	207	296	146	169
T/2L	51	HI	199	118	273
2L	51	174	120	342	74
H/B/RL	52	156	124	125	151
		Cage 2			
H/2L	37	172	147	112	487
H/A	64	187	213	403	433
H	59	83	135	322	520
RL	103	132	83	284	HI
T	77	348	173	96	175
A	55	184	220	271	HI

CALCULATIONS SHOWING AMOUNTS OF STZ, CITRATE BUFFER, EXTRACT GIVEN TO EACH GROUP

1. Normal control group:

The rats were given citrate buffer 1ml/kg body weight only. The volume given to each rat was calculated using the formula below:

1ml = 1000g

Xml = weight of rat

Therefore, Xml = $\frac{1ml \times weight\ of\ rats}{1000g}$ …**eqn 1**

Table of normal control citrate buffer

Name	Buffer (ml)
H/B	0.104
T	0.110
B	0.102
H/T	0.102
H	0.107

2. Diabetic control group:

The rats were given the STZ (45mg/kg) dissolved in 0.1M citrate buffer (1ml/kg) solution. The volume of the solution given to each rat was calculated using this formula below:

The amount of STZ dissolved in buffer

45mg = 1ml

Xmg = Zml

Therefore Zml = 0.022Xmg …**eqn 2.1**

Amount of STZ used given per rat

45mg = 1000g

Xmg = weight of rats

Therefore, Xmg = $\frac{45mg \times weight\ of\ rats}{1000g}$…**eqn 2.2**

Where, X = amount of STZ in mg

Z = amount of citrate buffer in ml

Table of diabetic control rats STZ

Name	STZ/citrate buffer solution (ml)
Cage 1	
H/T	0.14
H/B	0.14
A	0.14
A/RL	0.15
2L	0.15
T/2L	0.15
H/2L	0.14
Cage 2	
H/T	0.13
H	0.14
B	0.15
H/A	0.14
B/T	0.13
H/RL	0.13

3. **Normal treated group:**

Buffer

The rats were given the citrate buffer 1ml/kg refer to eqn 1:

Extract

The rats were also given extract 400mg/kg body weight. The volume of extract given was calculated using the formula below:

The extract was dissolved in water (5% stock solution) 5g/100ml.

5g of extract = 100ml of water

400mg of extract = xml of water

Therefore 400mg = 8ml of water = 1000g rat

1000g = 8ml

Weight of rat = Eml

Therefore, Eml = $\frac{8ml \times weight\ of\ rat}{1000g}$…**equ 3**

Where, E= amount of extract solution for each rat

Values of each rat are represented below:

Name	Citrate buffer (ml)	Extract dose (ml)
H/B	0.13	1.06
H/RL	0.13	1.03
H/T	0.12	0.98
B	0.13	1.03
A	0.12	0.96

4. **Diabetic treated group:**

These rats were given both the STZ solution, refer to **eqn 3** and the extract solution, refer to **eqn 2.1** and **eqn 2.2**

Value for each rat represented below:

Name	Citrate buffer / STZ solution (ml)	Extract (ml)
	Cage 1	
A	0.14	1.1
B/RL	0.15	1.2
T/2L	0.15	1.2
2L	0.14	1.1
H/B/RL	0.16	1.2
	Cage 2	
H/2L	0.14	1.1
H/A	0.16	1.3
H	0.16	1.2

RL	0.15	1.2
T	0.16	1.3
A	0.16	1.3